

REVIEW ON ASSESSING METHODS IN CHITOSAN BASED POLYSACCHARIDE COMPOUNDS PREPARATIONS AND THEIR DERIVATIVE ACTIVITIES

M. K. CHANDAN & P. B. RAMESH BABU

Department of Genetic Engineering, Bharath Institute of Science and Technology, Selaiyur, Chennai, India

ABSTRACT

Chitin is a most commonly occurring natural polysaccharide, which is processed by industries into various active compounds of biological importance. The best suitable method is deacetylation, which produces chitosan from chitin. In recent biodiversity and natural conservation approaches, waste water treatment is one important aspect of renewable natural resources, in which the separation of colloids from waste water is processed through coagulation-flocculation processes. There were several recent methods have been reported in separating squid gladius chitin and found that deacetylation process is the most economical and viable process in chitosan extraction from chitin. The potency of chitosan as anti-pollution activity has been tested using bromophenol blue in various laboratory conditions. In the present study, we report using 0.01% bromophenol blue as anti-pollutant agent. It was demonstrated that chitosan as a suitable candidate for cleaning water in waste water treatment plants.

KEYWORDS: Chitin, Chitosan, Adsorption, Water treatment & Anti-pollution activity

Received: May 10, 2021; **Accepted:** May 31, 2021; **Published:** Jul 02, 2021; **Paper Id.:** IJBTRDEC20212

INTRODUCTION

The most common and naturally available resource for Chitin is a natural polymer, initially described by French Professor, Henri Braconnot, in 1811. Chitin was reported as the liberally available biopolymer after cellulose with an annual production of 10^{10} to 10^{11} tons¹. Considering the biological and economical importance it was reported that chitin is having similarity to another polysaccharide compound namely cellulose and chitin itself is described as an derivative of cellulose in which structural modifications were made using C2 hydroxyl groups, which is known to be changed with acetamido residues^{1,2}. The physical-chemical property of chitin is described as microfibril crystals, which forms the basis in structural organization of many living organisms. It is generally described that Chitin is a substance for spatial and bioactivity in several organisms to withstand natural forces or as a protection in predatory surroundings.

Increasingly chitin is found valuable importance in commercial applications with industrial product value which are used as a soluble derivative in industrial biotechnology, cosmetics, animal husbandry, nutraceuticals, textile industries, agriculture, food industries, paper manufacture etc. Recently the commercial value of chitin has been exploited worldwide considering its low cost availability in natural sources. In several organisms, chitin is present as ubiquitous polymers in cell walls of algae, fungi and in insect cuticles, shells of crustaceans and molluscan shells. In animal kingdom, several sources were described for chitin and mostly in lower plants and invertebrates. In fungi, it was described as a well-known major component in shells and exoskeletons of crustacean and insects. Being a major polymer in several organisms and by weight chitin is reported as approximately 75% of total living component in arthropods and others. It is reported that nearly 10,000 tons of

shellfish waste are available every year, that supports the production of raw material for making chitin. The structural component or skeletal component of chitin includes amino sugars, comprising of two monomeric units namely N-acetylglucosamine and glucosamine.

The structural configuration of chitin consist of linear unbranched chains of β -(1 \rightarrow 4) linked 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) residues of polysaccharides (Figure 1). The quantity of carbohydrate polymer (glucosamine) present in low in quantity in chitin and which is reported less soluble in solvents and water³¹. The β -1,4-linkage between the monomeric units constitute a straight chain structure, reliable and hard structured into chitin. The commonly found and distributed hydroxyl groups and amino groups of the repeated monomers consists of inter and intra molecular hydrogen bonds which form straight association of repeated monomers in congregation with elaborate and intertwined crystallinity³². The physic chemical mass (in molecular weight) of chitin can be as high as 10^6 Da and the spatial configuration of chitin is shown in [Figure 1](#). In naturally occurring environmental resources, chitin is diversified in different polymeric forms namely α , β and γ with various physic-chemical properties³³. The different structural configuration of chitin exhibit in their arrangement of the polymeric chain ([Figure 2](#)). In α -chitin, the polymeric units are present in anti-parallel to each other, in β -chitin, they are present parallel to each other and in γ -chitin the polymeric chains are present uneven in which two parallel chains and one anti-parallel chain develops into polymeric structure.

In natural resources of chitin, crustaceans such as crabs and shrimp largely contain α -chitin, whereas squids constitute β -chitin and γ -chitin is from loligo³². The degree of modification by deacetylation of chitin and molecular size and weight of chitin is dependent on methods of preparation and based on the chitin source material obtained. The variability and the extent of deacetylation can be specified as the molar fraction of chemical reaction of monomer units existing in higher order structure of chitin polymers and their natural resources.³⁴. The chemical isolation method by deacetylation makes the chitin product as chitin or chitosan. Such physico-chemical analysis classifies chitin if DDA is less than 50% and the product is termed chitosan if the DDA is greater than 50%³⁵. Therefore deacytlation process is the crucial physico-chemical facto which leads to the final product of chitin properties viz. solubility, flexibility, polymer conformation and viscosity³⁶.

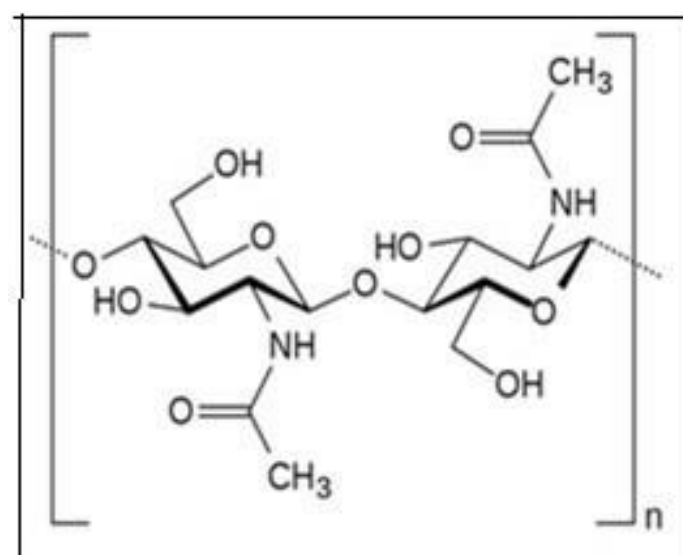


Figure 1: Chemical structure of Chitin

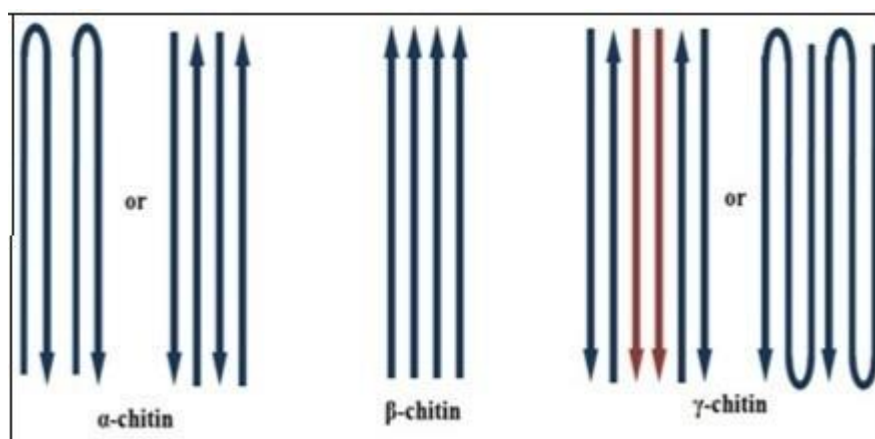


Figure 2: Schematic representation of three different Polymeric Configurations (α , β and γ) of chitin

In conventional methods of chitin extraction, chemical treatment of crustacean exoskeletons constitutes mixer of processes by which proteins are degraded, demineralized and bleaching of the material. Proteolytic degradation is carried out by treating chitin materials with alkaline sodium hydroxide solution potassium hydroxide³⁷. Demineralisation is usually done by taking fresh exoskeleton or molluscan shells and treating by lower the pH with hydrochloric acid, Nitric acid and sulphuric acids at a high temperature of 90-100°C³⁸. Finally, bleaching is done for decolorisation to get colourless chitin. As a substitute method, it is also separation using a few biological methods by proteolytic degradation using proteases obtained from various microbial sources by replacing methods using alkaline solutions.

The most commonly used methods using biological degradation of chitin resources from natural sources include using proteolytic enzymes and using probiotic microorganisms obtained from natural sources. The earlier method of extraction using microbial proteases was previously reported as the most effective technique than the isolating chitin by chemical methods⁴⁰. In chitin extraction methods using biological processes, both removal of minerals by demineralization and proteolytic degradation occur simultaneously. Another classical method of crustacean family shell waste chitin production include fermentation technology using shell wastes of shrimp (*Penaeus monodon*). In a few other methods, lactic acid bacteria were used to recover chitin by including carbohydrates as a natural energy source⁴¹. In figure 3, the method applied using enzymatic technology in isolating chitin is schematically represented.

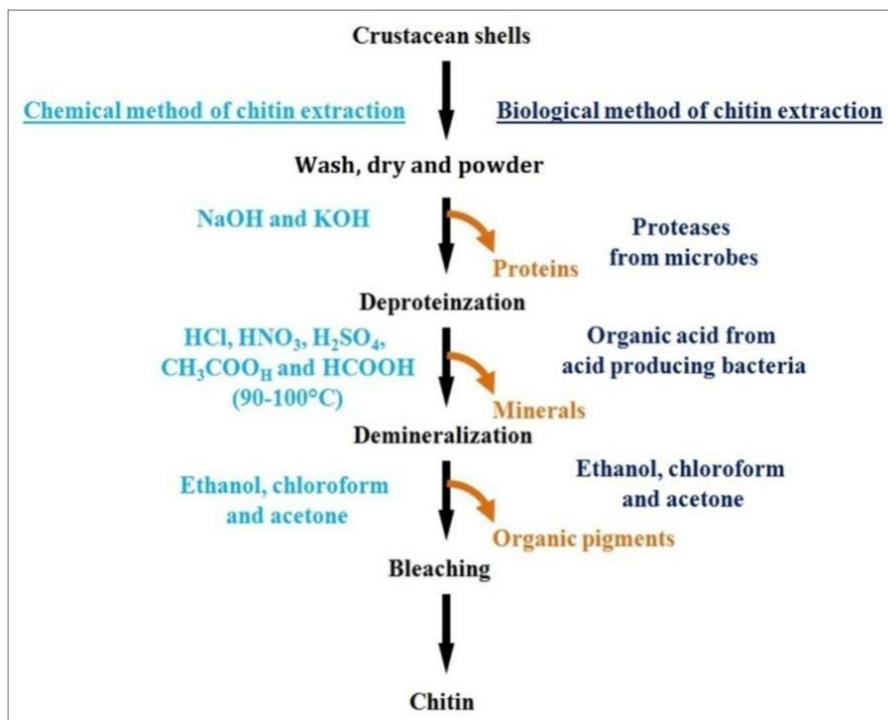


Figure 3: A schematic representation of the chemical and biological (enzymatic) methods for Chitin Extraction

The various applications of chitin is largely depended on physico-chemical properties such as solubility, porosity, and surface area⁴¹⁻⁴⁵. In order to avoid the problems associated with chitin extraction and problems or improper chitin treatment could result in various byproducts or chitin derivatives. As it is well described by several authors, the major utility of chitin is dependent on chitosan preparation. The physico-chemical method plays an important parameter in chitosan production in industries, alkaline hydroxide processing of chitin is performed at 100 °C. The amount of alkaline component (NaOH) concentration and influence of thermal conditions in chemical processing are known to influence the production yield of chitosan from natural resources. The solubility of the chitosan products can be determined using DDA content, the final product of chitosan may be soluble in water or mild acidic solution⁴⁷.

Water Treatment

The most valuable application chitosan is in waste water treatment plants such as textiles, leather, paper, and plastics, contain several kinds of synthetic dyestuffs. A minute quantity of dye in water is more clearly seen and can be deleterious to organisms in water and toxic to humans. Therefore, separating the dye component from chitosan from waste water effluent systems are identified as fundamental importance to the natural habitat. The reported adsorption characteristics of chitin and its derivative are highly promising and useful as decontaminating agents and depletion of harmful materials and dyes¹¹. Prado et al., 2004 analysed the comparison of chitosan adsorption properties of indigo carmine dye and chitosan. Their findings described adsorption of chitosan than chitin due to the existence of alkaline nitrogen centers in chitosan and indigo carmine dye¹⁴. Dolphen et al., (2007) compared the adsorption properties of chitin derivatives and chitin modified with sodium hypochlorite solution in Reactive Red 141 from wastewater. The hydroxyl group of the modified chitin was converted into CH_2OCl which failed to react with the dye solution. Hence, dye adsorption by modified chitin characterized mainly by physical adsorption and adsorption capacity, which was higher than that of chitin⁴². A few other investigators reported chitin was converted into pure chitin hydrogel (CG3), which produced very good mechanical properties and

biological compatibility, for wastewater treatment. CG3 exhibited microporous structure, large surface area and affinity on malachite green, leading to the high uptake capacity of dye¹³.

Methods in Chitin extractions from crustaceans and evaluation of antipollution activity.

Various crustaceans and arthropods have found importance in evaluating antipollution activities. Cephalopods constitute one such major source in evaluation procedures and as described in earlier methods demineralization and proteolytic degradation play important steps in preparing chitin derivatives and testing anti-pollution activities. Initial steps in such methods including washing the gladius and preparing the dried material in a hot air oven for 6 hours then wash it again in the flowing water after that dry the sample in a hot air oven for 12 hrs. After that pulverise the gladius using a pestle and mortar. The next step is to prepare the powder samples which is treated with acids such as HCl for one day, in order to get rid of the protein content. Later the materials are subjected to alkaline treatment with 1N NaOH at 80°C for 24 hours to remove protein. The finally separated chitin derivative component as chitosan was processed through deacetylation process in 40% aqueous NaOH by heating under reflux for 6 hours at 110°C and was cooled at room temperature. Then the precipitate was observed which was washed with appropriate buffered solution and kept at room temperature for 12 hours with stirring in 10% acetic acid solution. The pH was adjusted to 10 with 40% NaOH solution. The solution was dialyzed against deionized water for 24 hours. The product was centrifuged at 10,000rpm for ten minutes and lyophilized to give chitosan.

Evaluation of anti-pollution activity is carried out using powdered form well grounded until fine powder obtained. Next study to find the adsorption of the bromophenol blue on top of powder surface was determined by observing the absorbance of Bromophenol blue dissolved in water at 590 nm. Such reaction mixture was kept in light protected area to allow adsorption of bromopenol blue on powder surfaces. Later on, various dosage of sample was used to check the adsorption of Bromophenol blue and samples of 1 mL were extracted after 1hr, the powders were separated from the liquid using centrifugation, and, then, readings were taken using a Cary-60 UV-Vis spectrophotometer at 590nm to assess the absorbance spectrum of the sample tested.

Table 4: Table of different Concentration of chitosan in mg, Spectrophotometer abs and % of adsorption

Concentration (mg)	Spectrophotometer abs	% of adsorption
control	0.498	0
100	0.015	96.98795
200	0.026	94.77912
300	0.048	90.36145
400	0.03	93.9759
500	0.104	79.11647

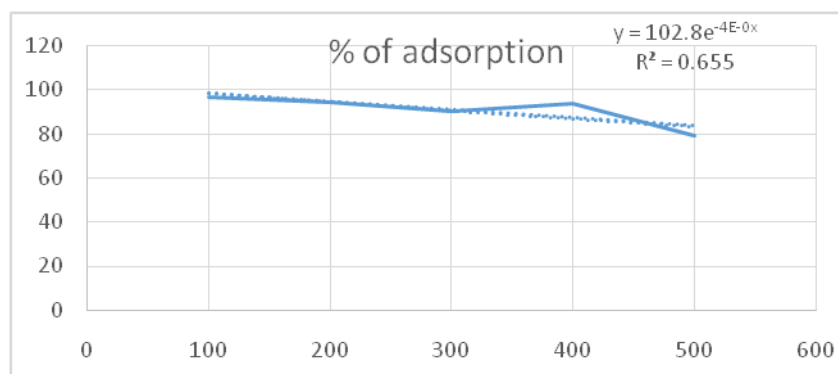


Figure 5: Exponential graph of % of adsorption of bromophenol blue over the surface of chitosan at different concentration

Our study reported a review of methods in extraction of chitosan and chitin derivatives based on the previous literature information. The most commonly used and most effective way of treatment in such a procedure is deacetylation process. The major utility of such product application is well exploited in evaluating the chitosan for anti-pollution activity at various dosages. As it is reported that 10ml Bromophenol blue (0.001%) in each test is found best suited to test as chemical pollutant. Based on a previous literature report, it was found at 590nm, abs readings were taken and an exponential graph was plotted, percentage of adsorption was recorded reduced as concentration increased (Figure 4 & 5). Based on these findings it could be concluded that chitosan can be found suitable naturally occurring resourceful material for water cleaning with minimal concentration range. In the future, chitosan will be tested for antipollution activity at a lower concentration to find out the optimal concentration for water treatment.

REFERENCES

1. Gooday G W. (1994) *Physiology of microbial degradation of chitin and chitosan*. In *Biochemistry of microbial degradation*, Springer Netherlands 279-312
2. Rinaudo M. (2006) *Chitin and chitosan: properties and applications.*, *Prog Polym Sci* 31, 603-632.
3. Józef S, Nadia A A. (2003) *Production, Properties, and Some New Applications of Chitin and Its Derivatives.*, *Crit Rev Food Sci Nutrition* 43, 145-171.
4. K. Naveena Latha, P. Ram Reddy & D. V. Ravi Shanka, "Non Chemical Water Treatment Process for TDS Reduction In Cooling Tower – Specific Study on Electrical Conductivity and Turbidity ", *International Journal of Mechanical and Production Engineering Research and Development (IJMPERD)*, Vol. 8 Issue 6, pp, 151-158
5. Revathi M, Saravanan R. Shanmugam A (2012), *Production and characterization of chitinase from Vibrio species, a head waste of shrimp. Metapenaeus dobsonii (Miers, 1878) and chitin of Sepiella inermis Orbigny, 1848*. *Adv Biosci Tech* 4, 392-397.
6. B. O. Akinuli, E. A. Fadiji, P. P. Ikubanni & O. O. Agboola, "Decision Support System (DSS) For Domestic Well-Water Treatment Plant Installation", *International Journal of Mechanical and Production Engineering Research and Development (IJMPERD)*, Vol. 9, Issue 6, pp, 673-676
7. Kuddus M, Ahmad I Z. (2013) *Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase.*, *J Genet Eng Biotechnol* 11, 39-46.
8. Xu Y, Bajaj M, Schneider R, Grage S L, Ulrich A S et al. (2013) *Transformation of the matrix structure of shrimp shells during*

- bacterial deproteination and demineralization., *Microb Cell Factories* 12, 90-102.
9. G. Gnanavel & P. Muthusamy, "Pharmaceutical Industry Waste Water Treatment using Atmospheric Air and Pure Oxygen", *IASET: International Journal of Metallurgical, Materials and Chemical Engineering (IASET: IJMMCE)* Vol. 7, Issue 6, pp; 1-6
 10. Gabius H J. (2011) *The sugar code: fundamentals of glycosciences*. John Wiley & Sons., United States
 11. Zhang M, Haga A, Sekiguchi H, Hirano S. (2000) Structure of insect chitin isolated from beetle larva cuticle and silkworm (*Bombyx mori*) pupa exuvia., *Int J Biol Macromol* 27, 99-105.
 12. Liu S, Sun J, Yu L, Zhang C, Bi J et al. (2012) Extraction and characterization of chitin from the beetle *Holotrichia parallela motschulsky*., *Molecules* 17, 4604-4611.
 13. Nemtsev S V, Zueva O Y, Khismatullin M R, Albulov A I, Varlamov V P. (2004) Isolation of chitin and chitosan from honeybees., *Appl Biochem Microbiol* 40, 39-43.
 14. Suresh Shukla & M. A. Singaracharya, "Bacteria and its Good Benefits in Chemical Process Industry Effluents Treatments", *International Journal of Applied and Natural Sciences (IJANS)*, Vol. 7, Issue 2, pp; 111 - 118
 15. Farnesi L C, RFS Menna-Barreto, Martins A J, Valle D, Rezende G L. (2015) Physical features and chitin content of eggs from the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*: Connection with distinct levels of resistance to desiccation., *J Insect Physiol* 83, 43-52.
 16. Harkin C, Brück W M, Lynch C. (2015) Isolation & identification of bacteria for the treatment of brown crab (*Cancer pagurus*) waste to produce chitinous material., *J Appl Microbiol* 118, 954-965.
 17. Shanmugam K A. (2016) Modified Process for deproteination of green crab shells (*Carcinus maenas*) extraction of chitin/chitosan., *J Chem Pharm Sci* 9, 163-165.
 18. Sorokoumov I, Zagorskiy I, Zagorskaya D, Uryash V, Kokurina N et al. (2014) Physicochemical properties of chitin isolated from shell of industrial crabs of various species. *Progress on Chemistry and Application of Chitin and its Derivatives* 15, 5-10.
 19. Percot A, Viton C, Domard A. (2003) Optimization of chitin extraction from shrimp shells., *Biomacromol* 4, 12-18.
 20. Puvvada Y S, Vankayalapati S, Sukhavasi S. (2012) Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry., *Intl Curr Pharm J* 1, 258-263.
 21. Solairaj D, Rameshthangam P, Srinivasan P. (2016) Adsorption of methylene blue, bromophenol blue, and coomassie brilliant blue by α -chitin nanoparticles., *J Adv Res* 7, 113-124.
 22. Cahú T B, Santos S D, Mendes A, Córdula C R, Chavante S F et al. (2012) Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (*Litopenaeus vannamei*) processing waste., *Process Biochem* 47, 570-577.
 23. Auerswald L, Gäde G. (2008) Simultaneous extraction of chitin and astaxanthin from waste of lobsters *Jasus lalandii*, and use of astaxanthin as an aquacultural feed additive., *Afr J Mar Sci* 30, 35-44.
 24. Raabe D, Al-Sawalmih A, Yi S B, Fabritius H. (2007) Preferred crystallographic texture of α -chitin as a microscopic and macroscopic design principle of the exoskeleton of the lobster *Homarus americanus*., *Acta Biomaterialia* 3, 882-895.
 25. Rhazi M, Desbrieres J, Tolaimate A, Alagui A, Vottero P. (2000) Investigation of different natural sources of chitin: influence of the source and deacetylation process on the physicochemical characteristics of chitosan., *Polym Intl* 49, 337-344.
 26. Hartmann M H, Kaplan D L. (1998) Biopolymers from renewable resources., *Kaplan, DL*, Ed 367, 629-638.

27. Muzzarelli R A, Ilari P, Tarsi R, Dubini B, Xia W. (1994) Chitosan from *Absidia coerulea*., *Carbohydr Polym* 25, 45-50.
28. Kumaresapillai N, Basha R A, Sathish R. (2011) Production and evaluation of chitosan from *Aspergillus niger* MTCC strains., *Iranian J Pharm Res* 10, 553-558.
29. Synowiecki J, Al-Khateeb NAAQ. (1997) Mycelia of *Mucor rouxii* as a source of chitin and chitosan., *Food Chem* 60, 605-610.
30. Ruiz-Flores E, Lopez-Romero E, Gutierrez-Corona F. (1990) Chitin synthetase activity in a developmental mutant of *Phycomyces Blakesleeana*., *Antonie van Leeuwenhoek* 58, 67-72.
31. Maw T, Tan T K, Khor E, Wong S M. (2002) Selection of *Gongronella butleri* strains for enhanced chitosan yield with UV mutagenesis., *J Biotechnol* 95, 189-193.
32. Vaingankar P N, Juvekar A R. (2014) Fermentative Production of Mycelial Chitosan from Zygomycetes: media optimization and physico-chemical characterization., *Adv Biosci Biotechnol* 5, 940-956.
33. Ramanathan A, Kittusamy R. (2011) Antihepatotoxic effect of isolated chitin from *Rhizopus oryzae* against paracetamol-induced hepatotoxicity., *Bangladesh J Pharm* 6, 64-67.
34. Messner R, Kubicek C P. (1990) Synthesis of cell wall glucan, chitin, and protein by regenerating protoplasts and mycelia of *Trichoderma reesei*., *Can J Microbiol* 36, 211-217.
35. Vetter J. (2007) Chitin content of cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*., *Food Chem* 102, 6-9.
36. Khor E, Lim L Y. (2003) Implantable applications of chitin and chitosan., *Biomater* 24, 2339-2349.
37. Aranaz I, Mengibar M, Harris R, Paños I, Miralles B et al. (2009) Functional characterization of chitin and chitosan., *Curr Chem Biol* 3, 203-230.
38. Mark H. (2004). *Encyclopedia of Polymer Science and Technology*, 12 Volume Set. Wiley-Interscience, New York.
39. Zhang Y, Xue C, Xue Y, Gao R, Zhang X. (2005) Determination of the degree of deacetylation of chitin and chitosan by X-ray powder diffraction., *Carbohydr Res* 340, 1914-1917.
40. Kasaai M R. (2009) Various methods for determination of the degree of N-acetylation of chitin and chitosan: a review., *J Agric Food Chem* 57, 1667-1676.
41. Dash M, Chiellini F, Ottenbrite R M, Chiellini E. (2011) Chitosan—A versatile semi-synthetic polymer in biomedical applications., *Prog Polym Sci* 36, 981-1014.
42. Percot A, Viton C, Domard A. (2003) Optimization of chitin extraction from shrimp shells., *Biomacromol* 4, 12-18.
43. No H K, Hur E Y. (1998) Control of foam formation by antifoam during demineralization of crustacean shell in preparation of chitin., *J Agric Food Chem* 46, 3844-3846.
44. Prameela K, Mohan C M, Smitha P V, KPJ Hemalatha. (2010) Bioremediation of shrimp biowaste by using natural probiotic for chitin and carotenoid production an alternative method to hazardous chemical method., *Int J Appl Biol Pharm Technol* 1, 903-910.
45. Khanafari A, REZA Marandi, Sanatei S. (2008) Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods., *J Environ Health Sci Eng* 5, 1-24.
46. Jung W J, Jo G H, Kuk J H, Kim Y J, Oh K T et al. (2007) Production of chitin from red crab shell waste by successive fermentation with *Lactobacillus paracasei* KCTC-3074 and *Serratia marcescens* FS-3., *Carbohydr Polym* 68, 746-750.

47. Synowiecki J, Al-Khateeb NAAQ (2000), *The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp Crangon crangon processing discards.*, *Food Chem* 68, 147-152.
48. Bautista J, Jover M, Gutierrez J F, Corpas R, Cremades O et al. (2001) *Preparation of crayfish chitin by in situ lactic acid production.*, *Process Biochem* 37, 229-234.
49. Adour L, Arbia W, Amrane A, Mameri N. (2008) *Combined use of waste materials—recovery of chitin from shrimp shells by lactic acid fermentation supplemented with date juice waste or glucose.*, *J Chem Technol Biotechnol* 83, 1664-1669.
50. Sudha P N. (2010) *Chitin/chitosan and derivatives for wastewater treatment.* In SK Kim (Ed.), *Chitin, Chitosan, Oligosaccharides and Their Derivatives: Biological Activities and Applications*, CRC Press, 561-585.
51. Mohammed M H, Williams P A, Tverezovskaya O. (2013) *Extraction of chitin from prawn shells and conversion to low molecular mass chitosan.*, *Food Hydrocoll* 31, 166-171.
52. Barikani M, Oliaei E, Seddiqi H, Honarkar H. (2014) *Preparation and application of chitin and its derivatives: A review.*, *Iranian Polym J* 23, 307-326.
53. Kurita K, Mori S, Nishiyama Y, Harata M. (2002) *N-Alkylation of chitin and some characteristics of the novel derivatives.*, *Polym Bull* 48, 159-166.
54. Zou Y, Khor E. (2009) *Preparation of sulfated-chitins under homogeneous conditions.*, *Carbohydr Polym* 77, 516-525.
55. Blasinska A, Drobnik J. (2008) *Effects of nonwoven mats of Di-O-butyrylchitin and related polymers on the process of wound healing.*, *Biomacromol* 9, 776-782.
56. Castagnino E, Ottaviani M F, Cangiotti M, Morelli M, Casettari L et al. (2008) *Radical scavenging activity of 5-methylpyrrolidinone chitosan and dibutyryl chitin.*, *Carbohydr Polym* 74, 640-647.
57. Bhatt L R, Kim B M, Hyun K, Kang K H, Lu C et al. (2011) *Preparation of chitin butyrate by using phosphoryl mixed anhydride system.*, *Carbohydr Res* 346, 691-694.
58. CKS Pillai, Paul W, Sharma C P. (2009) *Chitin and chitosan polymers: Chemistry, solubility and fiber formation.*, *Prog Polym Sci* 34, 641-678.

